Piglets Born after Vitrification of Embryos Using the Open Pulled Straw Method

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Morulae and unhatched blastocysts from Large White hyperprolific (LWh) and Meishan (MS) gilts were selected to test an ultrasapid open pulled straw (OPS) vitrification method with two media. The viability of vitrified/warmed embryos was estimated by the percentage of embryos that developed to the hatched blastocyst stage in vitro or by birth after transfer. In Experiment 1, two cryoprotectant dilution media were compared for cryopreservation of MS and LWh blastocysts: TCM was a standard Heps-buffered TCM199 + 20% NBS medium and PBS was a PBS + 20% NBS medium. After a two-step equilibration in ethylene glycol, dimethyl sulfoxide, and sucrose, 1-5 blastocysts were loaded into OPS and plunged into liquid nitrogen. Embryos were warmed; a four-step dilution with decreasing concentrations of sucrose was applied. In PBS, LWh blastocysts (27%) had a lower viability in vitro than MS blastocysts (67%; P = 0.001). In TCM, no significant difference was observed between genotypes (41% for LWh and 43% for MS blastocysts) and both viability rates were lower than that of the control group. In Experiment 2, morula-stage LWh and MS embryos were vitrified and warmed using PBS. The viability rate was low and did not differ between LWh (11%) and MS (14%). In Experiment 3, 200 MS and 200 LWh blastocysts were vitrified/warmed as described in Experiment 1 (PBS). In each of 20 MS recipients, 20 embryos were transferred. The survival rate was 55% and recipients farrowed four and five piglets (median) for MS and LWh blastocysts, respectively. The OPS method is therefore appropriate for cryopreservation of unhatched porcine blastocysts. 

Key Words: pig; vitrification; OPS; morula; blastocyst; genotype; in vitro developement; embryo transfer.

Porcine embryos are known to be more sensitive to damage caused by cryopreservation than other mammal embryos (7, 26). Only a few piglets have been born after transfer of slow-rate frozen and thawed embryos (21, 22). The presence of a large number of intracytoplasmic lipid droplets has often been mentioned as an obstacle to successful freezing of porcine embryos. Removal of these lipid droplets may increase survival rates: three piglets were born after the transfer of 181 delipidated frozen/thawed embryos (23). However, none of the methods proposed have given satisfactory results. Moreover, they are often difficult to implement. In addition, most of the results achieved have not been confirmed in later experiments. Conventional freezing therefore does not seem to be suitable for preservation of porcine embryos.

Vitrification is currently being studied in several species to resolve the problems of cryopreservation. To totally eliminate ice crystal formation, embryos are equilibrated with high concentrations of cryoprotectants and then plunged immediately into liquid nitrogen. Using traditional 250-μL insemination straws, the fastest achievable cooling rate is approximately 2500°C/min, which allows embryos to pass through certain critical temperature zones quickly and decreases chilling injuries (27). With pigs, this method has so far resulted in the birth of 10 piglets after transfer of 157 cytoskeletal-stabilized and vitrified/thawed hatched blastocysts. Twenty-nine piglets were also born after the transfer of 224 cytoskeletal-stabilized hatched blastocysts (8, 9). Another team obtained 11 piglets after 17 transfers of 16 to 30 expanded and hatched blastocysts (12, 13). However, only 1 successful transfer (4 piglets)
of exclusively unhatched blastocysts has so far been reported (13).

Several new techniques have recently been developed to increase the cooling and warming rates of vitrification, including the use of electron microscopic grids to provide support to oocytes and embryos during manipulations (19, 31) and open pulled straw (OPS) technology. The low volume of cryoprotectant medium surrounding oocytes or embryos and the direct contact with liquid nitrogen provide very high cooling rates (approx 24,000°C/min with OPS technology). These methods have been successfully used for vitrification of oocytes and embryos in cattle, and high in vitro survival rates were achieved after cryopreservation of embryos from several other species, including pigs (11, 15, 19, 31, 33, 35). The faster cooling rate seems to be one of the key solutions to protecting porcine embryos from chilling injuries and obtaining piglet births after vitrification of unhatched blastocysts (1, 2). Our preliminary results (2) confirm this observation.

In this study, we attempted to confirm the efficiency of rapid cooling performed with OPS vitrification (33, 35). Two cryoprotectant dilution media were compared to determine whether saline concentration or osmotic pressure had an effect on the blastocyst survival. As differences in embryonic development have been observed in Meishan and Large White or crossbred embryos (10, 32), we tested these two cryoprotectant media on Meishan (MS) and Large White hyperprolific (LWh) embryos to ensure that the method used was not specific to a given genotype. To complete the study, the most efficient cryoprotectant media were used for vitrification of morula-stage Meishan and Large White hyperprolific embryos. Finally, one of the methods was selected to evaluate in vivo blastocyst development of these two genotypes after vitrification, warming, and transfer to recipients.

MATERIALS AND METHODS

Animals

Embryo donors and recipients came from the INRA experimental pig herd in Nouzilly, France. They were cyclic gilts aged 5 to 8 months at the time of their introduction into the experiments. Donors were Meishan genotype (n = 62) or Large White hyperprolific genotype (n = 63) (5). Recipients were Meishan (n = 20).

Embryo Production and Collection

Embryo production. Estrus detection was checked twice per day with a boar. Embryo donors underwent double insemination at an interval of 12–24 h at a spontaneous estrus. The semen collections and doses were prepared by the INRA Experimental Artificial Insemination Center (Station Expérimentale d’Insémination Artificielle, Rouillé, France). Donor females were inseminated twice with 3 × 10⁶ spermatozoa per insemination, using semen from a boar of a different genotype (mostly Piétrain) to benefit from heterosis (16). The maternal genotype of the embryo is indicated only when discussing embryo genotypes.

Embryo collection. Donor gilts were slaughtered 5 to 6 days after the first insemination, and their reproductive tracts were immediately removed. Embryos were collected after flushing the uterine horns with phosphate buffer at 39°C containing 2% newborn calf serum (NBCS) from GIBCO-BRL, France. Embryo development stages were evaluated under a stereomicroscope, with 20× magnification. Only morulae and/or unhatched blastocysts were selected.

Embryo culture and survival evaluation. In vitro development of control and vitrified/warmed embryos was carried out at 39°C in an atmosphere containing 5% CO₂, 5% O₂, and 90% N₂ in 100 µl of culture medium, without polyvinyl pyrrolidone but with 20% fetal calf serum (FCS) added (GIBCO–BRL) (3). This medium was adapted from UB medium, derived from NCSU23 medium (24). FCS was always used instead of NBCS for in vitro culture of embryos. The duration of culture was 2 to 5 days, depending on the initial stage of the embryo.

Only embryos that hatched after a maximum of 5 days were considered surviving. The hatch-
TABLE 1  
Experimental Outline

<table>
<thead>
<tr>
<th>Exp.</th>
<th>Genotype</th>
<th>Stage</th>
<th>T (°C)</th>
<th>Medium (Med)</th>
<th>Vitrification</th>
<th>Time (min)</th>
<th>Warming</th>
<th>Time (min)</th>
<th>Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L.Wh MS</td>
<td>Unattached Blastocyst</td>
<td>39</td>
<td>TCM or PBSx1 + PBSx2</td>
<td>Med alone</td>
<td>1</td>
<td>Med + 0.2 M Suc</td>
<td>1</td>
<td>In vivo</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Med alone</td>
<td>1</td>
<td>Med + 0.2 M Suc</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Med + 1 M MeSO + 1.3 M EG</td>
<td>3</td>
<td>Med + 0.1 M Suc</td>
<td>5</td>
<td>Med alone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Med + 2.5 M MeSO + 3.2 M EG + 0.6 M Suc</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>L.Wh MS</td>
<td>Morula</td>
<td>39</td>
<td>PBSx1 + PBSx2</td>
<td>Med alone</td>
<td>1</td>
<td>Med + 0.2 M Suc</td>
<td>1</td>
<td>In vivo</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>Med alone</td>
<td>1</td>
<td>Med + 0.2 M Suc</td>
<td>5</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Med + 1.4 M MeSO + 1.8 M EG</td>
<td>3</td>
<td>Med + 0.1 M Suc</td>
<td>5</td>
<td>Med alone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Med + 2.8 M MeSO + 3.6 M EG + 0.8 M Suc</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>L.Wh MS</td>
<td>Unattached Blastocyst</td>
<td>39</td>
<td>PBSx1 + PBSx2</td>
<td>Med alone</td>
<td>1</td>
<td>Med + 0.2 M Suc</td>
<td>1</td>
<td>In vivo</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>Med alone</td>
<td>1</td>
<td>Med + 0.2 M Suc</td>
<td>5</td>
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<td>Med + 1 M MeSO + 1.3 M EG</td>
<td>3</td>
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<td>5</td>
<td>Med alone</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Med + 2.5 M MeSO + 3.2 M EG + 0.6 M Suc</td>
<td>1</td>
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</tr>
</tbody>
</table>

Note. L.Wh, Large White hyperprolific; MS, Meishan; Suc, sucrose; TCM, Heps TCM199 + 20% NBCS; PBSx1, PBS + 20% NBCS; PBSx2, PBS double concentration + 20% NBCS.

Experimental Protocol

An experimental outline for the three experiments is shown in Table 1.

Experiment 1: Effect of Vitrification Medium and Genotype of Blastocysts on Hatching Rate after In Vitro Development

Two cryoprotectant dilution media were tested to determine their effects on blastocyst survival for the two genotypes:

(a) PBS, which was a Dulbecco's phosphate-buffered saline medium adjusted to 290 mOsm using double concentration (PBS × 2) to compensate for the saline dilution due to the high concentration of cryoprotectants.

(b) TCM, which was a Heps-buffered TCM199 medium.

Both media were supplemented with 20% NBCS. The cryoprotectants used were dimethyl sulfoxide (Me₂SO) and ethylene glycol (EG) (35). These two media and the cryoprotectants were purchased from Sigma (France) and OPS was purchased from Szigfr. Co. (Clayton, Australia).

At vitrification, Large White hyperprolific and Meishan blastocysts were randomly allocated among the two media. Vitrification was performed using OPS in groups of two to five blastocysts. All equilibrations were performed at 39°C.

Blastocysts were equilibrated successively in two subsequent baths of TCM or PBS for 1 min and then in:

- Cryoprotectant 1: 1 M Me₂SO + 1.3 M EG diluted in TCM or PBS for 3 min.
- Cryoprotectant 2: 2.5 M Me₂SO + 3.2 M EG + 0.6 M sucrose diluted in TCM or PBS for 1 min.

Blastocysts were then drawn up together in a 2-μl drop of Cryoprotectant 2 medium measured with a pipette, gently placed in the bottom of a culture dish, and loaded into OPS with the narrow end. The OPS used was a French ministraw, heat-soften, pulled, and cut at the narrowest point to reduce the diameter at one end.
to approximately half of the original. Due to the capillary effect, the 2-μl drop with the embryos was immediately loaded into the straw and then submerged into liquid nitrogen (33).

After removal from the liquid nitrogen, straws were maintained in ambient air for 5 s before the narrow end was immersed in the first rehydration solution at 39°C. The embryos were gradually removed from the straw by gravity. The cryoprotectant dilution was made by passing the embryos through three successive dilution solutions:

- Rehydration 1: TCM or PBS + 0.2 M sucrose for 1 min.
- Rehydration 2: TCM or PBS + 0.2 M sucrose for 5 min.
- Rehydration 3: TCM or PBS + 0.1 M sucrose for 5 min and then for 5 min in TCM or PBS.

All blastocysts were then transferred to culture medium and cultured as described earlier to evaluate their capacity to hatch.

Experiment 2: Effect of Developmental Stage and Genotype

Morula-stage Large White hyperprolific and Meishan embryos were vitrified in this experiment, using PBS. The embryos were vitrified and warmed using the same procedure as that described for Experiment 1, but cryoprotectant concentrations were different from those in Experiment 1 and corresponded to those indicated by G. Vajta (personal communication).

Embryos were equilibrated at 39°C successively in the following media: twice in PBS for 1 min and then in

- Cryoprotectant 1: PBS + 1.4 M Me₂SO + 1.8 M EG for 3 min.
- Cryoprotectant 2: PBS + 2.8 M Me₂SO + 3.6 M EG + 0.6 M sucrose for 1 min.

Warming and embryo culture were performed as described for Experiment 1.

Experiment 3: Viability of Vitrified/Warmed Meishan or Large White Hyperprolific Blastocysts after Transfer

Large White hyperprolific (n = 200) and Meishan (n = 200) blastocysts, vitrified/warmed as described for Experiment 1 and without selection, were transferred to 20 Meishan recipients, 1 to 2 h after warming and subsequent in vitro culture (20 blastocysts to each recipient). Blastocysts were collected in 50 μl culture medium in a Teflon embryo catheter (diameter 1 mm) connected to a 1-ml syringe and then transferred into a recipient. Recipients were Meishan gilts, since it has been shown that gestation and embryo survival rates are higher with this genotype (20).

No recipients were inseminated. The transfer was performed surgically (16) and asynchronously (−24 h) with gilts relative to donors. This means that the onset of estrus of the recipients appeared 24 h after that of the donors. Embryos were transferred after a midventral laparotomy at the upper ends of one uterine horn, through a hole made in the wall of the uterus.

Gestation was assessed by ultrasonography around day 25 postestrus (18). Then, at the time of farrowing, the number of piglets born was recorded. Survival rate at farrowing was the ratio of the number of live-born piglets to the number of vitrified/warmed blastocysts transferred and is expressed as percentage.

Data Collection and Statistical Analysis

The number of corpora lutea, the number of embryos collected, the number of vitrified morulae and/or blastocysts, and the number of embryos hatched after in vitro culture were recorded for each donor. The ovulation rate and the number of embryos collected were analyzed using the GLM procedure of the SAS program (28). The interactions between treatments and genotypes on the hatching rate were analyzed by the CATMOD procedure of the SAS program (28). Pairwise comparison was made with the S-PLUS proportion test (30). Since a constant number of blastocysts was transferred to
TABLE 2  
In Vitro Hatching Rate for Large White Hyperprolific and Meishan Blastocysts According to Vitriﬁcation Medium

<table>
<thead>
<tr>
<th>Medium</th>
<th>Large White hyperprolific</th>
<th>Meishan</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCM</td>
<td>41%* (46) [7]</td>
<td>43%* (43) [7]</td>
</tr>
<tr>
<td>PBS</td>
<td>27%* (45) [6]</td>
<td>67%* (45) [6]</td>
</tr>
<tr>
<td>Control (nonvitrified)</td>
<td>70%* (40) [13]</td>
<td>72%* (1) [11]</td>
</tr>
</tbody>
</table>

Note. (n), Number of embryos; [n], number of replicates.  
* P = 0.001; †P = 0.02; ‡P = 0.21; §P = 0.97; ††P = 0.98.

TABLE 3  
In Vitro Hatching Rate for Large White Hyperprolific and Meishan Morulae

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Large White hyperprolific</th>
<th>Meishan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitrified/warmed</td>
<td>11.5%* (61) [9]</td>
<td>14%* (57) [9]</td>
</tr>
<tr>
<td>Control (nonvitrified)</td>
<td>64.5%* (17) [8]</td>
<td>72%* (11) [4]</td>
</tr>
</tbody>
</table>

Note. (n), Number of embryos; [n], number of replicates.  
* P = 0.41; †P = 0.0001.

RESULTS

Embryo Production and Collection

The ovulation rate was signiﬁcantly higher (P = 0.0001) in Large White hyperprolific gilts (17.4 ± 0.7; mean ± SE) than in Meishan gilts (14.5 ± 0.7). The number of embryos collected differed between genotypes: 12.9 ± 0.8 for Large White hyperprolific and 10.4 ± 0.8 for Meishan (P < 0.01). Average embryo collection rate was 74%, which was the ratio of the number of collected embryos to the number of corpora lutea.

Experiment 1: Effect of the Vitriﬁcation Medium and Genotype of Blastocysts on Hatching Rate after In Vitro Development

As shown in Table 2, a difference between genotypes was observed in hatching rates when PBS was used. The hatching rate for Meishan blastocysts was signiﬁcantly higher than that for Large White hyperprolific blastocysts (67% vs 27%, respectively, P = 0.001) and not signiﬁcantly different from that of the control Meishan blastocysts (67% vs 72%, respectively, P = 0.98) or from that of the control Large White hyperprolific blastocysts. For Meishan blastocysts, the hatching rate differed between PBS and TCM (P = 0.02). Results with TCM did not differ between the two genotypes. The hatching rate was 41 and 43% for Large White hyperprolific and Meishan, respectively (P = 0.97).

Experiment 2: Effect of Developmental Stage and Genotype

Vitriﬁcation at the morula stage was followed by a low in vitro survival rate, and there was no signiﬁcant difference between the two genotypes (LWh 11% vs MS 14%; P = 0.41). These rates were signiﬁcantly lower than those from the control group, both in Large White hyperprolific and in Meishan (Table 3; P = 0.0001).

Experiment 3: Viability of Vitriﬁed/Warmed Meishan or Large White Hyperprolific Blastocysts after Transfer

Overall, the farrowing rate was 55% for the 20 transfers but this parameter varied between the two genotypes, 80% in Meishan and 30% in Large White hyperprolific (Table 4), and the difference was signiﬁcant (P = 0.04). Five of

TABLE 4  
Results of Farrowings Obtained after Transfer (20 Vitrified/Warmed Blastocysts per Recipient)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of recipient gilts</th>
<th>Number of farrowed gilts</th>
<th>Farrowing rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large White hyperprolific</td>
<td>10 [9]</td>
<td>3</td>
<td>30%*</td>
</tr>
<tr>
<td>Meishan</td>
<td>10 [9]</td>
<td>8</td>
<td>80%*</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>11</td>
<td>55%</td>
</tr>
</tbody>
</table>

Note. [n], number of replicates.  
* P = 0.04.
10 recipients of Large White hyperprolific blastocysts became pregnant (Tables 4 and 6), and 3 gave birth to five, five, and two piglets (Table 5). The nonpregnant recipients returned to estrus within the normal expected period (19 days), with the exception of 2 that occurred at 29 and 39 days (Table 6). Ten transfers were performed with Meishan blastocysts, 9 recipients became pregnant, and 8 recipients farrowed from one to five piglets (Table 5). The 2 open recipients returned to estrus at days 20 and 27 postestrus (Table 6). Survival rate at farrowing was significantly higher in Meishan than in Large White hyperprolific: 13.5 and 5.5%, respectively ($P = 0.04$; Table 5). Piglets born after transfer were normal, and no anatomical abnormalities were observed after farrowing. Live-born piglets had birth and weaning weights within the normal range for these genotypes (data not shown).

**DISCUSSION**

The purpose of this study was to attempt to verify whether cooling rate was a key factor in porcine embryo vitrification. For species for which embryo vitrification is difficult, such as *Drosophila* spp. (31), an increase in cooling rate provided positive results. Faster cooling may be obtained by using a smaller volume of cryoprotectant to hold the embryos and by reducing the thickness of the straw wall, allowing closer contact with the liquid nitrogen. OPS technology provides both of these elements, which increased cooling rate approximately 10-fold compared to those achieved in standard straws (34). Vajta *et al.* (35) and Holm *et al.* (11) obtained higher *in vitro* hatching rates (73 and 71%, respectively) but only with Landrace embryos. *In vivo*, Beebe *et al.* (1) obtained piglets after centrifugation, cytoskeletal stabilization, vitrification, and transfer of early blastocysts.

Our studies indicated that other factors may also influence *in vitro* survival after vitrification. The developmental stage of the embryo at the time of vitrification seems to be important. Despite an increased cooling speed, the hatching rate after *in vitro* development for morulae remained low (Table 3) and confirmed previous studies showing that cryoconservation of pig embryos can be performed successfully either at hatched blastocyst stages (6) or by mixing blastocysts and hatched blastocysts (13). However, it is not established whether or not *in vitro* development of morulae is fully efficient (7, 9, 22).

Dilution media of cryoprotectants may also play an important role in cryoinjuries and survival. The hatching percentage of blastocysts vitrified/warmed with PBS was significantly lower than that with TCM for the Large White hyperprolific.
hyperprolific genotype. TCM199 medium contains many amino acids, which may act protectively, and the Hepes-buffered system, which may also have a favorable effect on Large White hyperprolific embryos. It has recently been shown that, for hamster embryos, cryopreservation reduces the ability to regulate intracellular pH (14). The PBS consisted of a phosphate buffer whose osmolarity was increased with a PBS × 2 concentration to compensate for the salt dilution resulting from the addition of a high concentration of cryoprotectants. The low in vitro survival rates (27%) obtained with this medium for the Large White hyperprolific genotype may be explained by the negative effect of the high concentration of NaCl (29), which is not detrimental for the Meishan. For Meishan blastocysts, the PBS medium resulted in much better survival rates, nearly identical with that of the control group: 67% vs 72% (Table 2).

For in vivo study, PBS was chosen because that medium was much better for the Meishan blastocysts; it has allowed us to obtain high farrowing rates compared to those of Large White hyperprolific (80% vs 30%). This point again emphasizes that embryo genotype is an important factor.

An in vivo study with 180 early blastocysts treated with cytochalasin, centrifuged, vitrified with OPS, and transferred resulted in five piglets being born (1). Survival rate was low (3%) and may be explained by the number of manipulations, by different experimental conditions, and/or by different genotypes.

Another experiment with OPS, conducted with an experimental outline similar to ours (11), did not result in piglets being born after transfer. However, several differences may have influenced these results:

- The time of equilibration in Cryoprotectant 2 was different: 1 min in our method vs 0.5 min in their method. The extended time in our study may allow better penetration of cryoprotectants.
- The genotypes of donors and recipients were different: Holm et al. (11) used Danish Landrace sows and Large White crossbred gilts.

An in vivo and in vitro difference between Meishan and Large White hyperprolific blastocysts has been observed after vitrification. Moreover, we have shown that embryonic development varies between Large White and Meishan (32). Furthermore, the genotype of the recipient can affect the efficiency of embryo transfer, a higher pregnancy rate was obtained with transfer to Meishan recipients (83%; n = 48) than with transfer to crossbred recipients (54%; n = 48) (20).

- The transfer time in our study was with the same time lag; recipient gilts were asynchronous (−24 h) compared to donors. In Holm et al. (11), asynchrony is difficult to evaluate because embryos were cultured in vitro 1 to 3 days before vitrification. Pregnancy rate was always higher when transfers were made to recipients in which the onset of estrus was either synchronous with that of donors or 1 to 2 days later (25).

Survival rate at farrowing was low for both genotypes, 13.5% in Meishan and 5.5% in Large White hyperprolific, but these results were in agreement with other reports for hatched blastocysts. Dobrinsky et al. (9) obtained 6% (n = 157) and 13% (n = 224) of piglets after transfer of cytoskeletal-stabilized vitrified hatched blastocysts, and Kobayashi et al. (13) obtained 6.3% (n = 64) after transfer of vitrified expanded and hatched embryos.

An increase in this survival percentage may be obtained by reducing the number of transferred embryos, as suggested, after the transfer of fresh embryos (4, 25). Most of the recipients in these studies were European breeds, and they received 11 or more unhatched embryos. The percentages varied between 26 and 65% (4, 25). Cameron et al. (4) concluded that the optimum number of embryos that should be transferred to each recipient was between 12 and 16. For Meishan gilts, the optimum ranged around 10 embryos per recipient (17).

CONCLUSIONS

The cooling and/or warming rate is a key factor in the preservation of porcine embryos.
Genotype also seems to be an important factor, since embryonic development is not the same among genotypes. The stage of development is also important, and our method was found to be uniquely efficient for cryopreservation of blastocysts in vitro.

The open pulled straw technology is a reliable and efficient method for cryopreservation of porcine unilaterally blastocysts. High pregnancy and farrowing rates can be achieved, opening a new possibility for the porcine embryo transfer industry. Moreover, the method is simple and easy to implement. It may become a new tool for conservation of genetic resources.

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